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SPECTROPHOTOMETRIC DETERMINATION OF 4-AMINOAZOBENZENES, 4-AMINOSTILBENES, SCHIFF BASES, AND ANALOGOUS COMPOUNDS WITH 3-METHYL-2-BENZOTHIAZOLONE HYDRAZONE

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SUMMARY

3-Methyl-2-benzothiazolone hydrazone is shown to be a powerful and versatile electrophilic reagent which can be used for the spectral detection and determination of strongly nucleophilic molecules containing a carbon atom of high electron density. The determination of approximately 50 azo dyes and a smaller group of stilbene and Schiff base derivatives by a new procedure for these compounds is described. 4-Amino-, 4-alkylamino, and 4-dialkylamino derivatives of azobenzene can be readily analyzed. The determination of N,N-dialkyl-4-aminoazobenzene was thoroughly investigated. Beer's law was obeyed from 1 to more than 70 μg of compound per 10 ml of final solution. The color was stable for approximately 1/2 hour. The molar absorptivity for N,N-dimethyl-4-aminoazobenzene was 70,000; the absorptivity was 0.31 μg^{-1} ml cm^{-1}. N,N-Dimethyl-p-aminostilbene reacted readily in the procedure; it had a molar absorptivity of 119,000 and an absorptivity of 0.53 μg^{-1} ml cm^{-1}. The procedure should prove of value in the detection and determination of two types of carcinogens, the aminoazobenzenes and the aminostilbenes.

INTRODUCTION

New analytical methods for various types of carcinogens have been introduced in previous papers, e.g. for benzo[a]pyrene (10,12), polycyclic aromatic hydrocarbons (8-10, 12, 14, 16, 18), primary aromatic amines (17,19), secondary and tertiary aromatic amines (19), and aromatic acylamines (7).

A very large number of derivatives of 4-phenylazoaniline (commonly called 4-aminoazobenzene or AB with the numbering shown in Table I) have been shown to be carcinogenic to animals (4). These compounds can be detected and determined by means of their ultraviolet-visible absorption spectra in various solvents (6). They can be spectrally characterized by their basicity (1,11,15) and their C_a/A_a ratios (5), i.e. the relative proportion of the absorbances at the long wave length maxima of the 2 tautomers (C and A) obtained in acid solution.

Recent research has shown that many 4-aminostilbene derivatives are carcinogenic to animals (2). The ultraviolet absorption spectra of these amines have been reported (2).

However, in the trace analysis of a complex mixture for a 4-aminostilbene or a 4-aminoazobenzene, analysis through the ultraviolet-visible absorption spectra of the pure compound would be more difficult to apply. With the more sensitive 3-methyl-2-benzothiazolone hydrazone procedure as an additional tool these amines could be readily detected or determined in fairly complicated mixtures.

EXPERIMENTAL

Reagents and Equipment. 3-Methyl-2-benzothiazolone hydrazone hydrochloride was prepared (13) from commercially available 2-methylthiobenzothiazole.

The hydrazone salt is also available from the Aldrich Chemical Co., Milwaukee 10, Wisconsin. The azo dyes, stilbene derivatives, and other analogous compounds were prepared by standard literature procedures and crystallized from appropriate solvents to a constant melting point.

A Cary Model 11 recording spectrophotometer with 1 cm cells was used in the spectral work.

All values in Tables I and II are based on a minimum of two determinations and are within ± 2% of the average. The intensity values for N,N-dimethyl-4-aminoazobenzene (commonly called DAB) are based on 36 determinations giving a value of 70,000 ± 1,000 at wave length maximum 604 mμ. The value for N,N-dimethyl-4-aminostilbene at a wave length maximum of 667 mμ was 119,000 ± 3,000. The curves in Figures 3,4,5, and 6 are based on triplicate runs.

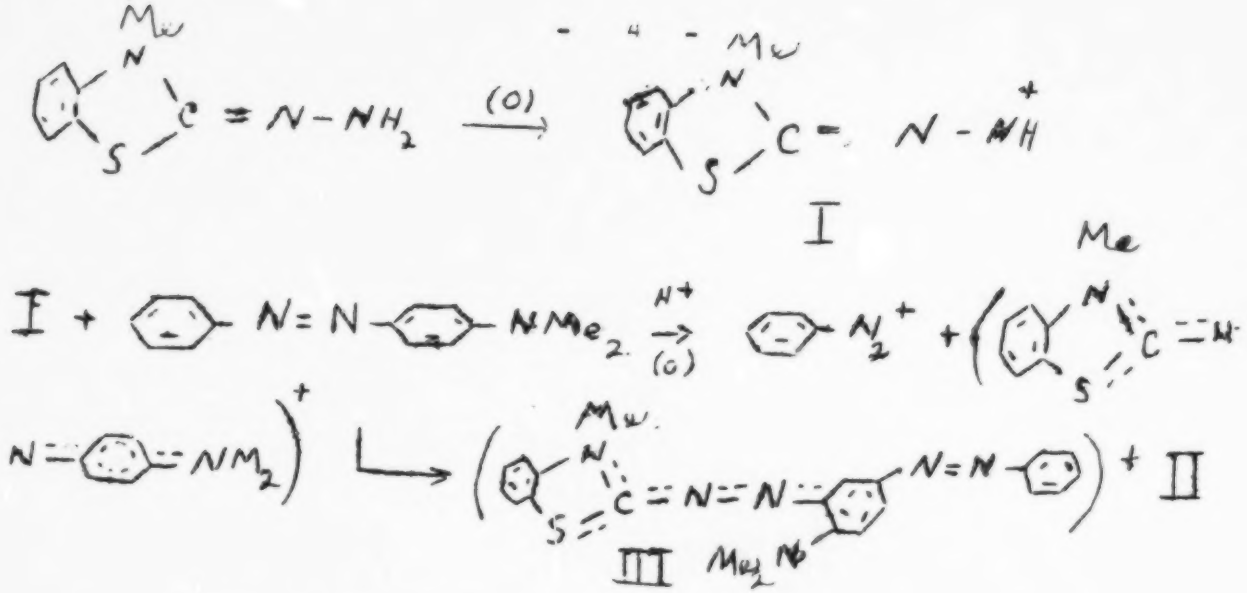
Reagent Solutions. 3-Methyl-2-benzothiazolone hydrazone hydrochloride, 0.2% aqueous solution. Stable for at least a week.

Ferric chloride, 1.3% aqueous solution.

Spectrophotometric Procedure. To 1 ml of the methanolic test solution was added 1 ml of the hydrazone reagent followed by 2 ml of the ferric chloride solution. The mixture was allowed to stand for 15 minutes and was then diluted to 10 ml with methanol. The absorption spectrum was determined within 20 minutes for DAB and within 5 minutes for the remainder of the test compounds. The wave length maxima and molar absorptivity values of a large number of compounds are given in Tables I and II.

RESULTS

Mechanism of Reactions. The mechanism in the formation of the blue chromogen is believed to involve the following reactions, as shown for DAB.



Dimethylaniline reacted with 3-methyl-2-benzothiazolone hydrazone by this procedure gives chromogen II, λ max. 598 mμ, ε 83,000 (see also 3,18). Consequently, the band at approximately 600 mμ obtained in the determination of DMAB is believed to be derived from chromogen II (Fig. 1), while the band at 672 mμ is believed to be derived from the chromogen III, obtained by substitution of the reagent in the ortho position to the dimethylamino group. In the same way N-methyl-AB and N-methylaniline, reacted with the reagent, should and do give a band at approximately 580 mμ while AB and aniline should and do give a band at approximately 570 mμ (Table I).

In a previous paper (18) spectral evidence was given for the postulate that many para substituted N,N-dialkylanilines are attacked by 3-methyl-2-benzothiazolone hydrazone at the para position by replacement of that particular group and/or at the ortho position. Attack of the reagent at the ortho position was postulated to account for the presence of bands at approximately 635 and 670 mμ. This latter type of band system can be seen in the absorption spectra obtained from N,N-dimethyl-4-aminostilbene and N,N-dimethyl-p-nitrosoaniline (Fig. 2). In the determination of N,N-dimethyl-4-aminobenzalaniline the spectrum

showed bands at 580, 625, and 670 mμ, which indicated the presence of two chromogens formed from splitting of the molecule at the CH = N band into aniline and N,N-dimethyl-4-aminobenzaldehyde. The aniline was attacked by the reagent in the para position and gave the band near 580 mμ. The original benzaldehyde derivative was attacked in the position ortho to the amino group. The bands at 625 and 670 mμ were derived from this chromogen. All the Schiff bases reacted in this manner. The same bands were obtained as were found in the separate fragments on reaction with the reagent.

Determination of DAB. Variables in the standard procedure for the determination of DAB were investigated. Attempts to decrease the dilution factor (e.g. 1 ml of test solution diluted to 10 ml of final volume) so as to increase the sensitivity resulted in turbid solutions. Optimum results were obtained with 0.15 to 0.20% of the hydrazone reagent (Fig. 3). In the oxidation step maximum absorbance was obtained at 1.2 to 1.4% ferric chloride solution (Fig. 3). Before the final dilution, the reaction mixture contained approximately 25% methanol. If the percentage of methanol was doubled in this 4 ml of mixture, the molar absorptivity was halved. The amount of methanol could not be decreased because of solubility problems with the test substance. The oxidation time was approximately 10 minutes for the attainment of maximum intensity (Fig. 4). The color intensity was stable for approximately 1/2 hour. From an initial value of 1.74, the absorbance dropped 0.01 units every hour and a half so that after 19 hours of standing the absorbance was 1.62. If the final diluent was concentrated hydrochloric acid instead of methanol, the solution was completely decolorized.

Beer's law was obeyed from 1 to more than 70 μg of DAB per 10 ml of final solution (Fig. 5). The molar absorptivity was 70,000 ± 1,000. The absorptivity

obtained for DAB in the procedure was 0.31 μg^{-1} ml cm^{-1}. One part of this dye could be detected in 360,000 parts of test solution. The spectral detection limit for this dye in the standard spectrophotometric procedure was approximately 3 µg. This figure could be decreased by using proportionately smaller volumes.

Determination of Azo Dyes. Approximately 50 azo dyes were determined by the standard procedure developed for DAB. To obtain optimum results for any other compound in Tables I and II conditions would have to be varied to some extent. Most 4-amino, 4-alkylamino, and 4-dialkylamino azobenzenes gave intense blue to green colors in the procedure, Table I. Derivatives of 2-methyl-DAB gave very high intensities. The characteristic absorption spectrum obtained with this particular type of compound displayed a strong band of aproximately 590 mµ and a shoulder at approximately 670 mµ with one-fourth to one-seventh the intensity of the main band.

In some cases either steric hindrance or substitution in the extended para position of a 4-aminoazobenzene had an adverse effect on the determination of that particular compound. The following compounds gave negative results in the test: azobenzene, 4-hydroxyazobenzene, 4-methylmercaptoazobenzene, and 4'-nitro-N,N-diethyl AB. The following compounds gave a weak reaction shown by a band at approximately 630 or 670 mµ with a molar absorptivity less than 4,000: 4'-nitro-N-phenyl AB, 4'-phenyl-DAB, 4'-nitro-N-methyl-N-ethyl-AB, 3-methyl-DAB, and N,N-dimethyl-p-(p-2-tolylazo-2-tolylazo) aniline. The compounds --- 4'-acetyl-DAB, 4'-methylmercapto-N-phenyl-AB, 2',4',6'-tribromo-DAB, 4'-thiocyano-DAB, and 2'-methoxycarbonyl-2-methyl-DAB did not react completely for they showed the presence of a substantial amount of unreacted material. The non-reactivity

of 3-methyl-DAB is in line with the very weak reaction of N,N-dimethyl-o-toluidine with the reagent.

Determination of Azo Dye Analogs. Approximately 25 compounds of this type have been reacted (Table II). Only a few stilbene type derivatives were available for investigation. Of these compounds, N,N-dimethyl-4-aminostilbene gave an extremely intense reaction with a molar absorptivity of 119,000 and an absorptivity of 0.53 μg^{-1} ml cm^{-1}. As sensitive methods for the trace analysis of aminostilbene compounds are not available in the literature, this procedure should fill that need.

Various types of Schiff bases gave positive results in the procedure (Table II). Consequently, the methods could be used for the detection, characterization, and determination of Schiff bases.

Negative results were obtained for the following compounds -- 1,1-dimethylhydrazine, 2-methylaminoethanol, malonic acid, hexamethylenetetramine, N-methyl-2-pyrrolidone, β-bromostyrene, piperonylic acid, 1-methyl-1-phenylhydrazine, N,N'-methylene-bis-acrylamide, 2-methylmercaptobenzothiazole, benzaldehyde, p-hydroxybenzaldehyde, and 4-hydroxybiphenyl.

3-Methyl-2-benzothiazolone hydrazone has been used for the determination of aliphatic aldehydes, aromatic amines, polycyclic imino heteroaromatic compounds, and azulene derivatives, so that any of these types of compounds could interfere in the determination of azo, stilbene or Schiff base compounds.

Another family of compounds that reacted readily with the reagent was the pyrrole derivatives. With pyrrole, wave length maxima of 600 and 664 mμ and molar absorptivities of 16,000 and 34,000, respectively, were obtained. N-Methylpyrrole gave bands at 612 and 673 mμ with molar absorptivites of 37,000 and

49,000, respectively, while 2,5-dimethylpyrrole gave equally intense bands at 635 and 657 mμ with a molar absorptivity of 28,000.

It must be emphasized that because of the general utility of the reagent, care must be exercised in the detection or determination of any particular compound or group of compounds that other interfering chemicals are not present, can be masked, or can be separated from the entity being determined.

DISCUSSION

3-Methyl-2-benzothiazolone hydrazone complements piperonal chloride (16) as a useful general reagent for organic compounds. This latter compound has been used in the analysis for aromatic and heteroaromatic compounds which have their highest electron density at an aromatic carbon atom and are more basic than benzene. It has been used as a measure of air pollution in different communities and at diverse sources. The hydrazone reacts with strongly nucleophilic molecules containing a carbon atom of high electron density. The application of this new general reagent to the field of air pollution trace analysis has been described in previous papers. Further possibilities are presented in the present paper. If necessary, the selectivity of the procedure could be considerably increased by the usual preliminary treatment, e.g. steam distillation, acid extraction, etc. In addition the reagent should be of value in the fields of cancer research, dye analysis, clinical chemistry, industrial air analysis, and as an intermediate in organic syntheses. The many vividly-colored dyes found capable of preparation in this and previous papers should be synthesized and studied for possible industrial application.

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LIST OF FIGURES

Figure 1. Visible absorption spectra obtained in standard procedure: dimethylaniline (2.77×10^{-5}M)(----) and N,N-dimethyl-4-aminoazobenzene (2.85×10^{-5}M) (_____).

Figure 2. Visible absorption spectra obtained in standard procedure: N,N-Dimethyl-p-nitrosoaniline (_____), N,N-Dimethyl-p-aminobenzalaniline (---), and N,N-Dimethyl-4-aminostilbene (.....).

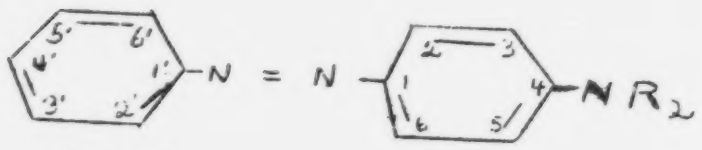
Figure 3. Effect of the concentration of 3-methyl-2-benzothiazolone hydrazone in the reagent solution and of ferric chloride in the oxidizing reagent on the absorbance obtained at λ max 605 mμ in the determination of 4-dimethylaminoazobenzene (2.4×10^{-5}M) by the standard procedure.

Figure 4. Effect of the oxidation time in minutes on the absorbance obtained at λ max 605 mμ in the determination of 4-dimethylaminoazobenzene (2.5×10^{-5}M) by the standard procedure.

Figure 5. Effect of the concentration of 4-dimethylaminoazobenzene on the absorbance at λ max 605 mμ obtained in the standard procedure.

TABLE I. - SPECTROPHOTOMETRIC DETERMINATION OF

4-AMINOAZOBENZENE DERIVATIVES



Compound	λ max, mμ	ε x 10^{-3}
4-Aminoazobenzene = AB	571	14
	644s	5
N-Methyl AB	589	36
	630	34
	664s	32
3'-Methyl-N-methyl AB	586	36
	625s	34
	664s	32
N-Ethyl AB	605	67
	666s	53
N-Phenyl AB	623	39
4'-Methylthio-N-phenyl AB	624	9
N,N-Dimethylaminoazobenzene = DAB	604	70
	672	56
2-Methyl DAB	588	72
	664s	20
2'-Amino DAB	608	45
	660s	37
2'-Chloro DAB	613	73
	664s	64
2'-Ethyl DAB	603	67
	665s	47
2'-Methoxy DAB	605	64
	665s	51
2'-Methyl DAB	603	69
	674s	45

TABLE I (cont.)

Compounds	λ max, mμ	ε x 10^{-3}
3'-Acetamino DAB	605	70
	664s	55
3'-Amino DAB	588	50
	672s	25
3'-Chloro DAB	608	66
	665s	54
3'-Ethoxy DAB	603	70
	663s	54
3'-Methyl DAB	603	68
	664s	52
3'-Nitro DAB	610	54
	665s	43
3'-Trifluoromethyl DAB	608	69
	663s	57
4'-Acetyl DAB	606	25
	666s	20
4'-Amino DAB	585	41
	668s	14
4'-Ethoxy DAB	598	49
	665s	29
4'-Ethyl DAB	603	59
	662s	43
4'-Fluoro DAB	603	37
	664s	26
4'-Methyl DAB	601	45
	666s	31
4'-Sulfo DAB	612	57
	666s	49
4'-Thiocyano DAB	599	18
	668s	12
2,2'-Dimethyl DAB	588	71
	665s	15

TABLE I (cont.)

Compound	λ max, mμ	ε x 10^{-3}
2-Methyl-2'-methoxycarbonyl DAB	583s	28
	665s	8
2,3'-Dimethyl DAB	588	71
	670s	14
2-Methyl-3'-chloro DAB	588	71
	665s	19
2-Methyl-4'-acetyl DAB	587	65
	666s	13
2-Methyl-4'-methylthio DAB	585	74
	670s	10
2',5'-Dimethyl DAB	601	66
	655s	43
2',4',6'-Tribromo DAB	612	5
	674s	4
N-Methyl-N-ethylaminoazobenzene = MEAB	603	58
	663s	32
2'-Chloro MEAB	605	63
	659s	51
2'-Nitro MEAB	613	42
	665s	34
3'-Acetamino MEAB	603	59
	659s	33
3'-Nitro MEAB	605	54
	660s	33
4'-Ethyl MEAB	600	60
	665s	29
4'-Fluoro MEAB	601	64
	663s	33
N-Methyl-N-benzyl AB	607	10
	678s	4
N,N-Diethyl AB	570s	32
	600	38
	674s	5

TABLE II. - SPECTROPHOTOMETRIC

DETERMINATION OF AZOBENZENE ANALOGS

Compound	λ max, mμ	ε x 10^{-3}
4-Phenylazo-1-naphthylamine	587	40
1-[N,N-Dimethyl-4-aminophenylazo]naphthalene	608	66
	662s	55
2-[N,N-Dimethyl-4-aminophenylazo]fluorene	573	12
	680s	4
N,N-Dimethyl-4-aminostilbene	632	116
	667	119
2-[N,N-Dimethyl-4-aminophenyl]-3-phenylquinoxaline	630	28
	665s	26
N-[4-Hydroxybenzylidene]aniline	571	54
	675	17
N-[N,N-Dimethyl-4-aminobenzylidene]aniline	580	84
	625	71
	670	69
2-Nitro-9-[N,N-Dimethyl-4-amino-N-benzylidene] fluorene	628	18
	667s	17
2-[N,N-Dimethyl-4-amino-N-benzylidene]-1,3-indandione	634	21
	668	21
N,N-Dimethyl-B,B-dicyano-4-vinyl aniline	637	56
	667	56
N[N,N-Dimethyl-4-amino-N-benzylidene]-p-nitro-aniline	630	38
	670	38
2[N-Benzylideneamino]fluorene	631s	36
	667	39
1[N,N-Dimethyl-4-amino-N-benzylideneamino]pyrene	637s	90
	677	102
	755s	49
2[p-Hydroxy-N-benzylideneamino]fluorene	625s	24
	670	28
N,N-Dimethyl-4-aminobenzaldehyde 2-quinolyl hydrazone	633	48
	665	47
N,N-Dimethyl-4-aminobenzaldehyde 2-benzothiazolyl hydrazone	555	37
	620	34
	668s	31